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**Evaluation of Proteins in Natural Rubber Latex Gloves and Pulmonary Function
amongst Female Nurses in Two Tertiary Hospitals in Southern, Thailand**

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Short running title

Latex glove exposure and lung function

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Structured abstract

Background: Inhaled natural rubber latex (NRL) allergens in a healthcare environment can cause NRL sensitization and reduce pulmonary functions.

Objectives: To determine the amount of proteins and the effects of NRL gloves on the pulmonary functions of female nurses in two hospitals in the southern Thailand.

Methods: The study included 340 female nurses from two hospitals in which self-reported information and a blood sample was collected. NRL sensitization was determined by using a solid-phase enzyme-labeled fluoroenzyme-immunoassay for anti-NRL IgE antibodies. Proteins in NRL gloves were measured by a modified Lowry method. Pulmonary function was measured by a spirometer.

Results: The prevalences of respiratory effects self-attributed to NRL glove use and of NRL sensitization were 6.5 and 4.7%, respectively. Four of the 16 sensitized nurses reported respiratory symptoms. NRL sensitized nurses had lower forced expiratory flow (FEF_{25-75%} predicted value) than those who were non-sensitized (Adj. difference = -12.56, 95% CI = -24.41 to -0.70). Furthermore, examination gloves contained protein levels in the range of 111 – 250 mg/dm². Different types of NRL glove contained with different amount of proteins. NRL sensitization was more prevalent in nurses with high concentrations of proteins in NRL gloves (p = 0.04).

Conclusion: Sensitization to NRL was associated with a decrease in FEF_{25 - 75%} predicted value, indicating narrowing of the small airways of the lung. Use of gloves with low proteins can reducing NRL allergen exposure in these hospitals, which may reduce the risk of developing respiratory problems and NRL sensitization.

Keywords: sensitization, pulmonary function, nurse, protein, glove

Introduction

Using powdered latex gloves (PLGs) at work can result in Healthcare workers (HCWs) having a high risk of exposure to latex allergens.¹ Cornstarch powder on PLGs has been reported as a natural rubber latex (NRL) allergen carrier.² NRL allergens in powdered gloves can spread out as airborne particles in workplaces during the wearing and removing of PLGs.³ A high concentration of NRL allergenic proteins has been found in NRL gloves; particularly in PLGs.^{1,4} In hospitals where a high concentration of NRL aeroallergen was detected, HCWs were more likely to have symptoms, including runny nose, wheezing, shortness of breath, rhinitis, rhino-conjunctivitis and asthma⁵⁻⁸ with a prevalence ranging between 2.3-4.8% of HCWs.^{7,8}

NRL allergy remains an occupational health concern, particularly in HCWs in developing countries such as Thailand due to the high cost of alternatives such as synthetic and powder-free gloves.^{9,10} In 2017, a study of NRL glove used in a university hospital in Thailand reported that PLG was mostly used in health care workers (95.6%), while less than 3 percent of them used nitrile gloves, powder free gloves and neoprene gloves.⁸ Furthermore, numerous medical instruments, such as surgical and examination gloves, catheters, blood pressure cuffs, tourniquets, stethoscopes, elastic bandages and wraps, containing NRL are also used in hospitals^{4,11} and high levels of NRL protein allergens have been reported in medical gloves, particularly PLGs.^{12,13} HCWs who wear PLGs can be exposed to NRL protein allergens by cutaneous and/or respiratory routes.¹⁴⁻¹⁶ Such exposure can induce respiratory symptoms in NRL sensitized individuals.¹⁷

Changes in pulmonary function (e.g. a reduced FEV₁) have been described.¹⁸ Respiratory reactions have been reported to occur even in those workers who no longer wear NRL gloves, but who stayed in the environment where PLGs were used.⁶ In a study amongst HCWs in the USA, NRL sensitized HCWs were more likely to leave employment within 2

years when compared with HCWs who were not sensitized to NRL.¹⁹ Hence there are significant health implications to the continuing use of PLGs in Thailand.

There is little information about the prevalence of health effects and on risk factors specifically related to NRL allergy in Thai HCWs, and so the aim of this study was to evaluate the amount of crude water soluble proteins in NRL gloves used in hospitals and to determine the effects of occupational use of NRL gloves on the pulmonary function and NRL sensitization of nurses in two tertiary hospitals in Thailand.

Methods

Study design and population.

An analytical observational study was conducted among 664 female nurses working in two tertiary hospitals in the south of Thailand. All nurses wore NRL gloves at work. The study was approved by the hospital human right committees from two hospitals (Ref. YL0027.102/17050 and Ref. SK0027.103/93) and the ethics committee of The University of Manchester (ref. 08117). This study was approved by Thai Clinical Trial Registry (TCTR: 20190605004). The information sheet and consent form were distributed to nurses before the study began. Three hundred forty participating nurses had signed in the consent form to confirm that they all agreed to take part of this study.

Questionnaire

Information on personal demographics (*i.e.* personal lifestyle), health status (*i.e.* history of ill-health, symptoms related to NRL gloves use) and occupational factors (*i.e.* working conditions and use of NRL gloves) was collected using a self-completed questionnaire. This questionnaire was translated to Thai language and back-translated into English by a second bi-

lingual researcher to ensure consistency and the clarity of translation. The content validity was considered by three experts. The index of item objective congruence (IOC) was 0.92.

Spirometry

Spirometry was measured using an electronic spirometer (Micro Medical MicroLab 3500, Cardinal Health, UK) using standard procedures and calibrated using normal values for the population of Thailand.²⁰ Before the test, personal information such as age, weight and height were recorded. Nurses were asked to sit in a convenient position, take a deep breath and blow into a spirometer. This was repeated 3 times, and the best of three acceptable blows (largest value) was recorded. The forced expiratory volume at one second (FEV₁) and forced vital capacity (FVC) were based on the maximal inspiration and expiration of the subjects. The forced expiratory flow (FEF)₂₅₋₇₅ was also recorded. The expected values were based on the age, sex and height of the subjects.

Detection of Anti-NRL Immunoglobulin E (IgE) Antibodies

Nurses provided 5 ml of blood. These samples were centrifuged (4,000g) at room temperature for 15 minutes, the serum separated and transferred into microcentrifuge tubes. Serum levels of anti-NRL IgE antibody was measured using a solid-phase enzyme-labelled fluoroenzyme immunoassay (NRL UniCAP Specific IgE Assay) in which allergens are covalently linked to high-binding-capacity sponge matrices. Specific IgE antibodies present in serum react with the NRL allergens and non-specific IgE antibodies are removed by washing the matrices. Enzyme labeled antibodies against IgE antibodies are then added, followed by developing reagents. Fluorescence was measured and antibody levels determined using a calibration curve. A positive IgE antibody on NRL was a result > 35 kU_A/I.²¹

Determination of protein levels in NRL Glove Extracts

Two types of NRL gloves most used in medical procedures were selected for testing, namely powdered examination gloves (PEGs) and powdered surgical gloves (PSGs). Each type of NRL glove used in hospital 1 and 2 was produced by different glove companies. Crude water soluble proteins of these gloves were extracted and quantified by using a Modified Lowry method (the standard of The American Society for Testing Materials (ASTM) test D5712-2005 protocol).²² Briefly, proteins from PLGs were extracted into aqueous buffer solution for pH 7.4 \pm 0.2 and then precipitated with sodium deoxycholate to concentrate them and to separate them from water soluble substances. The proteins were then redissolved in alkali and quantified colorimetrically by a Modified Lowry method. Spectrophotometric measurement was performed at a wavelength of 750 nm. The concentrations of protein in NRL gloves were reported in microgram per gram ($\mu\text{g/g}$) of NRL glove. The sensitivity of this method is 50 $\mu\text{g/g}$.²³

Statistical Analysis

Exploratory data analyses were conducted using frequency tables. Continuous data were described using mean, median, standard deviation, minimum and maximum values. Chi-square tests were used to analyse the difference in categorical variables. Fisher's exact test was used to analyse for small cell counts ($n < 5$). Mann Whitney U tests were used to analyse data when the assumption of the t-test was not met. Analysis of covariance (ANCOVA) was used to analyse effects of sensitization to latex on pulmonary functions. Statistical significance was considered when p-values were lower than 0.05.

Results

Of 664 nurses, 340 (51.2% response rate) completed the questionnaires, underwent a blood test and also spirometry. Detectable levels of IgE antibodies to NRL were found in 4.7%

of participating (16 of 340 nurses). The demographics of this population were described in Table 1. There were border significant differences on age and work period between NRL sensitised and non-sensitised nurses ($p = 0.07$).

Respiratory symptoms and pulmonary function

Of 340 nurses, 22 nurses (6.5%) reported respiratory symptoms related to NRL gloves used. Of 16 nurses with NRL sensitization, 4 sensitised nurses (25%) had reported respiratory symptoms related to NRL gloves used. The respiratory symptoms included sneezing, rhinitis, shortness of breath, wheezing and asthma. NRL-sensitized nurses had lower $FEF_{25-75\%}$ predicted values than non-sensitized nurses (Adjusted difference = -12.56 (95%CI -24.41 to -0.70); Table 2).

Use of NRL glove and level of proteins in NRL gloves

The results showed that PEGs had higher proteins than PSGs and there were different amount of protein levels in PEGs and PSGs between hospital 1 and 2. The protein levels were found in PEGs in the range of 111.1 – 250.8 $\mu\text{g}/\text{dm}^2$, while the NRL sterile gloves were found proteins in the range of 115.1 – 203.9 $\mu\text{g}/\text{dm}^2$. Moreover, there were significant differences in the levels of proteins of PEGs and PSGs between hospital 1 and 2 (Mean difference (95%CI) = 139.76 (112.53 to 166.99) and 88.82 (64.19 to 113.44), respectively). According to the consequence above, high latex sensitisation was found in the hospital where NRL gloves with high protein levels were used (6.9% vs 2.4%, $p = 0.04$). Furthermore, the latex sensitised nurses working in hospital 1 where used NRL gloves with high proteins had reported respiratory symptoms, while the NRL sensitised nurses in hospital 2 where used NRL gloves with low protein levels had not found, as shown in Table 3.

Discussion

High levels of water soluble proteins were found PLGs, particularly PEGs. However, it has been showed that the low water soluble protein level was found in powder free examination glove, as a study in Germany had evaluated the amount of proteins from powdered free gloves using a modified Lowry method. The results showed that 13 of 18 powder free gloves contained protein between 7.1 and 92.3 µg protein/g, while another 5 gloves were found under detection limit.²⁵ The German technical standards for hazardous material (TRGS 540) has been recommended that protein levels of NRL glove should be less than 30 µg proteins/g.^{24,25} According to the results in this current study, level of proteins in PLGs was eight times higher than the recommend value.

The prevalence of NRL sensitisation was higher where PLGs of high protein content were used to suggest an association between the two. This result supported the findings of a previous study which reported a decrease of NRL sensitisation and allergic symptoms related to NRL glove use when low protein NRL gloves were used in hospital workplaces.²⁶ For the respiratory symptoms which had found in 4 sensitised nurses, all of them worked in the hospital 1 where NRL gloves with high water soluble protein levels were used, while sensitized nurses in Hospital 2 where NRL gloves with low water soluble protein level were used. This may assume that NRL glove with a high protein level had also had a high level of NRL allergens. As, a previous study found that PEGs has highly released an amount of NRL aeroallergens which 68% of released particles sized was in the range of particle meter between 2.5 and 10 micron, and also 56% NRL aeroallergens were carried on them.²⁷

Exposure to NRL allergens among sensitized nurses in this study was associated with a decline in pulmonary function, particularly the FEF_{25-75%} predicted value, while there was no evidence of effect on the other pulmonary functions. The FEF_{25-75%} is a parameter which is more reflective of small airways and sensitive indicator of symptomatic asthma.²⁸ Another

study on FEF₂₅₋₇₅ in Italy reported that the abnormal FEF₂₅₋₇₅ was an early marker of airflow limitation and it had also suggested that the FEF₂₅₋₇₅ can be a predictive marker of newly diagnosed asthma.²⁹ It also has been reported that the reduced FEF₂₅₋₇₅ (<65% of predicted value) was associated with bronchial hyperreactivity.³⁰ FEF₂₅₋₇₅ could be used to predict airway hyper responsiveness in adult patient with asthma. Low FEF_{25-75%} predicted value can show that there was decreased function in the small airways of the lung. However, FEF_{25-75%} predicted values are not specific for small airway diseases,³¹ but is a sensitive indicator for airway obstruction.³² FEF_{25-75%} predicted value is more sensitive than FEV₁ in detecting peripheral or small airway narrowing in adults³³ as well as identifying individuals with asymptomatic asthma.³⁴ A previous study on the clinical diagnosis of asthma and FEF_{25-75%} predicted value has been compared the baseline FEF_{25-75%} predicted value using the subsequent methacholine bronchial provocation test and the results on FEF_{25-75%} predicted value in patients with positive bronchial provocation test had significantly lower than patient with normal responsive airway ($t = 4.616$, $p < 0.001$).³⁵ The change of small airways may be one of initially presenting parameters of a respiratory reaction in NRL sensitized nurses who worked in the environment with PLG use.

In the past years, there had numerous articles about NRL allergy in health care workers and also the study of NRL allergens in NRL gloves and other NRL products. Various types of glove used had different levels of proteins. These proteins were contained with NRL allergens inducing NRL sensitization in the health care workers. In developing countries as Thailand, the PLGs were widely used in hospitals for a long time until now. There were few studies about NRL allergy from NRL gloves exposed in Thai health care workers and no report for the proteins extracted from NRL glove and NRL sensitization (anti-NRL IgE antibodies) in Thai nurses. This current study revealed more information on the NRL sensitization, amount of

glove proteins, respiratory symptoms and pulmonary functions in nurses working in governmental hospitals.

The limitations of this study were that the voluntary participation may have resulted in volunteer bias in that nurses who reported health problems may have been overrepresented compared to nurses who were healthy. Furthermore, the healthy worker effects may be found in this study due to some evidences showed that non-sensitized nurse are older and have worked longer than sensitized nurses. The sensitized nurses might change their job positions due to health problems related to gloves used before the study was done. This study collected the self-reported data of respiratory symptoms related to gloves used, but fixed choice questions may lack flexibility and forces to answer and also social desirability bias may occur. The self-reported symptoms may occur not only expose to NRL allergens, but also chemical and powder cornstarch from PLGs. The design of this study cannot derive causal relationship between pulmonary function and proteins of NRL glove exposure and NRL sensitization. Furthermore, the confounding factors such as secondary smoking, underlying lung disease or allergic diseases may affect the result of pulmonary function test.

In conclusion, there was moderate evidence that exposure to NRL airborne allergens in the healthcare sector can affect pulmonary function of sensitized workers as measured by FEF₂₅ – 75% predicted value. The evidences in this study revealed exposure to high level protein in NRL glove has affected the respiratory and the low FEF₂₅₋₇₅ % predicted value where found in sensitized nurses. This suggests that it is important to minimize the level of NRL aeroallergens in the workplace environment by using powder free gloves or synthetic latex free gloves, which can reduce the risk of respiratory ill-health in NRL sensitized HCWs.

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346 **Table 1. Characteristics of nurses**

Variable	All nurses (n = 340)	Latex sensitized cases (n = 16)	Non-latex sensitized cases (n = 324)	P-value
Age (years)				0.07 ^a
Mean ± SD	36.9 ± 8.3	33.1 ± 8.2	37.1 ± 8.3	
Median	35.0	33.0	35.0	
Min-Max	23.0 – 60.0	23 - 48	23 - 60	
BMI classification, n (%)				0.49 ^a
Mean ± SD	22.1 ± 3.4	22.3 ± 2.9	22.1 ± 3.4	
Median	21.4	22.4	21.36	
Min-Max	116.0-34.5	17.9-30.3	16.0 – 34.5	
Education, n (%)				1.00
Diploma	16 (4.7)	1 (6.3)	15 (4.6)	
Bachelor and over	292 (85.9)	14 (87.5)	278 (85.5)	
Ethnicity, n (%)				
Thai	269 (79.1)	14 (87.5)	255 (78.7)	0.54 ^c
Thai-Chinese/Malaysian	71 (20.9)	2 (12.5)	69 (21.3)	
Non-Smoker, n (%)	340 (100.0)	16 (100.0)	324 (100.0)	-
Personal history of allergic disease (Y/N, % yes)	134/206 (39.4)	5/11 (31.3)	129/195 (39.8)	0.494 ^b
Type of personal allergic disease				
Hay fever (Y/N, % yes)	36/304 (10.6)	2/14 (12.5)	34/290 (10.5)	0.682 ^c
Grass allergy (Y/N, % yes)	31/309 (9.1)	2/14 (12.5)	29/295 (9.0)	0.648 ^c
Eczema (Y/N, % yes)	56/284 (16.5)	1/15 (6.3)	55/269 (17.0)	0.487 ^c

Hives (Y/N, % yes)	91/249 (26.8)	3/13 (18.8)	88/236 (27.2)	0.573 ^c
Asthma (Y/N, % yes)	16/324 (4.7)	2/14 (12.5)	14/310 (4.3)	0.170 ^c
Autoimmune (Y/N, % yes)	5/335 (1.5)	0/16 (0.0)	5/319 (1.5)	1.000 ^c
Atopy (Y/N, % yes)	87/253 (25.6)	3/13 (18.8)	84/240 (25.9)	0.770 ^c
Family history of allergic diseases (Y/N, % yes)	153/187 (45.0)	8/8 (50.0)	145/179 (44.8)	0.680 ^b
Type of family allergic disease				
Hay fever (Y/N, % yes)	37/303 (10.9)	3/13(18.8)	34/290 (10.5)	0.398 ^c
Grass allergy (Y/N, % yes)	29/311 (8.5)	2/14 (12.5)	27/297 (8.3)	0.636 ^c
Eczema (Y/N, % yes)	65/275 (19.1)	2/14 (12.5)	63/261 (19.4)	0.746 ^c
Hives (Y/N, % yes)	84/256 (24.7)	3/13 (18.8)	81/243 (25.0)	0.769 ^c
Asthma (Y/N, % yes)	67/273 (19.7)	2/14 (12.5)	65/259 (20.1)	0.747 ^c
Autoimmune (Y/N, % yes)	9/331 (2.6)	1/15 (6.3)	8/316 (2.5)	0.355 ^c
Atopy (Y/N, % yes)	122/218 (35.9)	6/10 (37.5)	116/208 (35.8)	1.000 ^c
Position, n (%)				
Registered nurses	323 (95.0)	15 (93.8)	308 (95.1)	0.57 ^b
Practical or Technical nurses	17 (5.0)	1 (6.3)	16 (4.9)	
Workplace, n (%)				
OPD	31 (9.1)	0 (0.0)	31 (9.6)	-
ER	19 (5.6)	0 (0.0)	19 (5.9)	
ICU	60 (17.6)	1 (6.3)	59 (18.2)	
OR	50 (14.7)	2 (12.5)	48 (14.8)	
LR	23 (6.8)	3 (18.8)	20 (6.2)	
Sur	74 (21.8)	4 (25.0)	70 (21.6)	
Obs	33 (9.7)	4 (25.0)	29 (9.0)	

Med	50 (14.7)	2 (12.5)	48 (14.8)	
Work period				
Mean \pm SD	14.7 \pm 8.5	11.0 \pm 8.1	14.9 \pm 8.5	
Median	13.0	10.9	13.0	0.07 ^b
Min-Max	0.7 – 36.0	0.8 – 24.1	0.7 - 36	
Work shift, n (%)				
\leq 35 hours/week	133 (39.2)	5 (31.3)	128 (39.6)	0.61 ^b
> 35 hours/week	206 (60.8)	11 (68.8)	195 (60.4)	

347 ^aP-value by Mann-Whitney U Test; ^bP-value by using Chi-Square Test; ^cP-value by using

348 Fisher's Exact Test.

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Table 2. Comparison of pulmonary function in sensitized and non-sensitized female nurses

Variable	Sensitized (n = 16)	Non-sensitized (n = 324)	<i>P</i> -value	Adj.difference (95% CI)
	Mean \pm SD	Mean \pm SD		
FEV ₁ (L)	2.21 \pm 0.20	2.24 \pm 0.35	0.62 ^a	- 0.04 (-0.19 to 0.11)
FVC (L)	2.51 \pm 0.30	2.51 \pm 0.40	0.95 ^a	0.01 (-0.17 to 0.18)
FEV ₁ /FVC ratio	88.3 \pm 4.7	89.7 \pm 5.5	0.28 ^a	- 1.51 (- 4.23 to 1.21)
FEV ₁ (% predicted)	90.7 \pm 10.2	91.8 \pm 12.1	0.74 ^b	- 1.01 (-7.03 to 5.01)
FVC (% predicted)	90.3 \pm 11.5	89.0 \pm 12.3	0.69 ^b	1.26 (-4.86 to 7.37)
FEF _{25% -75%} predicted	83.3 \pm 17.0	95.8 \pm 23.8	0.04 ^b	- 12.56 (- 24.41 to -0.70)

^aAdjusted for age, height, and weight using ANCOVA; ^bAdjusted for weight using ANCOVA; FEV₁: Force expiration volume in one second; FVC: Force volume capacity; FEF: Force expiratory flow

Table 3. Comparison of number of NRL glove used, total proteins in NRL gloves, latex sensitisation and respiratory symptoms in latex sensitised nurses between hospital 1 and 2

Variable	Hospital 1 Mean \pm SD	Hospital 2 Mean \pm SD	Mean Difference (95%CI)	P-value
-Average number of NRL examination glove used per day	9.97 \pm 9.54	9.17 \pm 9.34	0.80 (-1.36 to 2.96)	0.467
-Average number of NRL surgical glove used per day	6.77 \pm 9.41	6.36 \pm 7.29	0.41 (-1.71 to 2.54)	0.698
-Average total protein of powdered examination gloves (ug/dm ²) \pm SD	250.8 \pm 16.00	111.1 \pm 5.60	139.76 (112.53 to 166.99)	<0.001
-Average total protein of powdered surgical gloves (ug/dm ²) \pm SD	203.9 \pm 13.00	115.1 \pm 8.20	88.82 (64.19 to 113.44)	0.001
-Latex sensitization(%)	6.9	2.4		0.048
-Respiratory symptoms in latex sensitized nurses (n=4)	4	0		-

P-value by Independent t test

